

## Original Research Article

### ~~FREQUENCY COMPARING OF~~ SPOT URINE PROTEIN:CREATININE RATIO AND- 24-HOUR URINARY PROTEIN ESTIMATIONS IN TYPE 2 DIABETES MELLITUS PATIENTS

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#### ABSTRACT

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**OBJECTIVE:** To determine the frequency of abnormal spot urine protein:creatinine ratio and 24-hour urinary protein in type 2 diabetes mellitus patients

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**MATERIAL AND METHODS:** This prospective hospital based clinical study was conducted in the Department of General Medicine, Liaquat University of Medical & Health Sciences, Jamshoro, overbetween atthe periods of six months from 10<sup>th</sup><sup>st</sup> May 2018 to 9<sup>th</sup> November 2019 through a consecutive sampling technique. All the patients having age more than 18 years of both gender, and type 2 diabetes mellitus were enrolled in this study. Twenty-four hour urine sample was collected into the container having 5mL of 10% thymol in isopropanol as a preservative for 24 hours. A urine sample of 2mL was also taken for evaluation of proteins. Total volume of urine was noted and calculation was done for 24 hours. The proteinuria  $\geq 300\text{mg/dl}$  in 24-hour urine sample was considered as significant proteinuria. Kappa statistics was used to find agreement between spot urine protein and 24 hours urinary protein.

**Comment [H3]:** what about Creatinine Estimation?

**RESULTS:** A tTotal of 95 patients were evaluated and their mean age was  $41.91 \pm 14.29$  years, with male predominance ( $n = 66, 69.4\%$ ). Average 24 hour urinary protein was

1216.99±949.51mg and spot-urinary evaluation of protein was 1919.12±2129.25mg. The agreement between spot urinary protein creatinine ratio and 24 hour urinary protein was found in 82.1% of cases through Kappa statistics and the calculated agreement between the two procedures was 0.975 which provides sufficient agreement to use spot urine protein:creatinine ratio in routine diagnosis of proteinuria.

**CONCLUSION:** The study have shown that the protein:creatinine ratio for a random urine sample might be used to rule out the presence of significant proteinuria as defined by a quantitative measure of the 24-hour urine protein excretion.

**KEY WORDS:** Microalbuminuria, Spot Urine P:C Ratio, 24-h Urinary Protein, Type 2 Diabetes Mellitus

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## INTRODUCTION:

Diabetes mellitus (DM) is a chronic metabolic disorder and affects almost every organ in a body through its microvascular and macrovascular complications (1). Patients with untreated diabetes mellitus are associated with higher rates of complications that cause significant morbidity and mortality. Which is why, this chronic disease has become a major public health concern and can be very damaging as well as [...????](#). According to the World Health Organization (WHO), DM affects more than 170 million people worldwide and current prevalence in Pakistan is 11.77%, and this number will rise to 370 million by 2030 (2). ~~In a~~ The study conducted by Gross JL has shown that almost 25% of the patients with diabetes mellitus eventually develop some sort of kidney disease (3).

Detection of kidney disease in a patient with diabetes mellitus is quite easy and a simple blood test (urea and creatinine) and a urine test may indicate renal function deterioration and presence of microalbuminuria (which is also the first clinical signs of renal dysfunction in patients with diabetes mellitus), ~~respectively~~ (4). In a study conducted by Chowta NK, ~~has it was~~ observed that the prevalence of microalbuminuria was 37% (5) while similar prevalence (34%) was observed in Karachi Pakistan (6). Microalbuminuria is rarely reversible but may progress to overt proteinuria in around one third of patients with type 2 diabetes mellitus as compared to

type 1 diabetes mellitus. As per definition, microalbuminuria is defined as persistent presence of urine albumin ranging between 30mg/day to 300mg/day, is labeled as microalbuminuria.

24 hour urine collection is required to ascertain the protein excretion throughout the day, which is contrary to the statement that concentrated urine is required for determination of urine protein creatinine ratio. Now, it is proven that, urine protein creatinine excretion is constant throughout the day and there is no need to do the collection of 24 hour urine if the kidney glomerular filtration (GFR) rate is steady (7, 8). While, on the other hands, some clinicians also use random collection of urine sample called or "spot" urine sample and some also use urine specific gravity for determination of a ratio (9). Fagerstrom P and colleagues (10) in his study have observed that albumin excretion is much less throughout the day when it was expressed as a ratio to creatinine or urine specific gravity. A number of papers are published on this subject in the western world but there is shortage of the such data in Pakistani population. Due to this paucity of data in my region, ambiguity, I would like to carry out this study. The study aims to establish the utility of spot voided urine samples for detection of proteinuria as a credible and time saving method.

#### **PATIENTS AND METHODS:**

This prospective hospital based clinical study was conducted in the Department of General Medicine, Liaquat University of Medical & Health Sciences, Jamshoro, between the over a periods of six months from 10<sup>st</sup> th May 2018 to 9<sup>th</sup> November 2019 through a consecutive sampling technique. All the patients having age more than 18 years of both gender, and type 2 diabetes mellitus were enrolled in this study after taking their informed consent. Exclusion criteria for this study was; presence of diseases which may cause bias in our study by causing

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proteinuria i.e.g. hypertension, nephrotic syndrome, acute renal failure, impaired renal function due to non-diabetic cause, and hyperlipidemia.

The data was collected on the predesigned proforma, which ~~was~~ includes patient demographics, relevant history, and relevant investigations. Twenty-four hour urine sample was collected by instructing the patients or their attendants to collect all urine into the same container having 5mL of 10% thymol in isopropanol as a preservative for 24 hours. A sample of 2mL was taken for evaluation of proteins. Total volume of urine was noted and calculation was done for 24 hours. The proteinuria  $\geq 300\text{mg/dl}$  in 24-hour urine sample was considered as significant proteinuria.

A random urine sample of 5mL was collected ~~on the~~ next day any time just before the analysis and after completion of 24 hour sample collection. The fasting blood glucose, glycated hemoglobin, urine creatinine, and urine protein was estimated by kit method using semiautomatic analyzer Hitachi 912. The fasting blood glucose and glycated hemoglobin were estimated to know the diabetic status of the patient. Protein-creatinine ratio was calculated by dividing the urinary protein concentration by urinary creatinine concentration. HbA1c was only done once at the beginning of study.

#### **DATA ANALYSIS PROCEDURE:**

Statistical package for social sciences (SPSS) version 21 was used for data analysis purpose.

Descriptive statistics including ed mean  $\pm$  standard deviation (SD) was calculated for continuous data, i.e., age, serum creatinine, twenty four hour urinary protein, HbA1c, urinary creatinine and urinary protein. Frequencies were calculated from the categorical data, i.e., gender, microalbuminuria, fasting blood sugar, random blood sugar, and protein:creatinine ratio. Kappa statistics was used to find agreement between spot urine protein and 24 hours urinary protein.

Effect modifiers were controlled through stratification of age, gender, HbA1C and applied chi square test taken  $p \leq 0.05$  as significant.

## RESULTS:

Total of 95 patients were evaluated and their mean age was  $41.91 \pm 14.29$  years, with range of 47 (18-65) years. Among them 66 were males and 29 were female patients. The overall mean ~~Hb1Ae~~ HbA1C was  $6.18 \pm 0.99\%$  with range of 3.40% (4.40 – 7.80%). Table No. 01.

Table No. 02 shows that average 24 hour urinary protein was  $1216.99 \pm 949.51\text{mg}$  with a minimum of 100mg and maximum of 2600mg. In spot-urinary evaluation the average protein was  $1919.12 \pm 2129.25\text{mg}$  with minimum 84mg and maximum 9450mg and the average creatinine was 1616.0mg with a minimum of 250mg and maximum of 4500mg.

The agreement between spot urinary protein creatinine ratio and 24 hour urinary protein was observed and it was found that with respect to clinical analysis the agreement was found with in 82.1% cases,- This was also presented in Figure No. 01. The agreement was statistically analyzed through Kappa statistics and the calculated agreement between the two procedures was 0.975 which provides sufficient agreement to use spot protein:creatinine ratio ~~in~~ routinely to diagnose proteinuria Table No. 02. ~~It was observed~~ showed that out of the total of 78 patients with agreement, 54 (69.2%) patients were male and 24 (30.8%) were female patients. Among 17 non-agreement patients, frequency of male and female was 12 (70.6%) and 5

(29.4%), respectively. There was no significant association found between agreement with gender using chi-square test.

It was observed within agreement patients that 30 (38.5%) patients were had  $\leq 6.0\%$  Hb1Ac???? and 48 (61.5%) patients had were  $>6.0\%$  Hb1Ac.???. Among non-agreement patients, 6 (37.9%) patients had were  $\leq 6.0\%$  Hb1Ac?? and 11 (64.7%) patients were had  $>6.0\%$  Hb1Ac. There was no significant association found between agreement with Hb1Ac???? groups using chi-square test.

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## DISCUSSION:

Migrants from Indo-Asian who had microalbuminuria are more prone to faster progression of kidney disease as compared to native Europeans but disease prevalence, risk factors associated with microalbuminuria, and associated clinical conditions associated is are still lacking.

Diabetes mellitus is the most common cause of microalbuminuria and usually it occurs in 20% - 40% of patients when with disease duration of more than 10 years, and then it progresses to proteinuria in around 20% - 50% of the patients after 5-10 years with average reduction in renal functions of about is around 10-15 ml/min/year. Detection of microalbuminuria is crucial in the management of patients and it also helps in determining the overall prognosis of such patients. Most common methods used for the screening of microalbuminuria in Pakistan are 24 hour urine collection and measurement of albumin to creatinine ratio in random urine sample.

In our study the most common age group was middle age and among them males were more common than females, 69.4% and 30.5%, respectively. ~~In~~ ~~An~~ epidemiological study conducted in Germany has shown different findings and found that people with more than 50 years were more common to have microalbuminuria (5). The age difference is not significant but that showed early onset of diabetes mellitus in our population. ~~This~~ could be due to multiple factors such as cousin marriages, ~~leads to early onset of such kind of disease,~~ presence of hypertension and diabetes mellitus in more common than their study subjects, and poor dietary habits are also linked with increased burden of kidney diseases in our country. Also, some of the authors also observed change in protein excretion from morning to evening and night; this variation could be from 100% to 500%. The proper scientific reason is still not clear but authors have assumed that multiple factors could be linked such as increase in water intake, physical activity, and diet (11-13).

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More than 82% of the patients in our study were found to have agreement between spot urinary protein creatinine ratio and 24 hour urinary protein. Same observation was ~~observed~~ noted by ~~the~~ Wahbeh and colleagues (14). However, in another study conducted by Lane C et al (7) ~~have it was~~ observed that the agreement between agreement between spot urinary protein creatinine ratio and 24 hour urinary protein is higher at lower levels, but when the 24- hour urine protein excretion extends exceeds more than 2.0gm , ~~that~~ ~~n~~ agreement become suboptimal.

#### THE LIMITATIONS OF THIS STUDY:

The limitations of the study are: low sample size and the difficulties in collection of 24 hours urine sample in the female patients. Further study is required with larger sample size to emphasize the hypothesis.

Study is confined to a single center ~~with~~ which targeted only local subjects, so it is not clear whether our findings can be generalized nationally and internationally. We tested only a single urine sample, while confirmed diagnosis of MA requires persistence on at least two out of three consecutive tests. Screening for albuminuria is also recommended in high-risk subjects.

Emphasis ~~zed~~ was on adequacy of urine collection and ~~it was advised~~ was advised appropriate recollection in cases of doubt was advised. Furthermore, we deleted samples containing values of 24 h urine volume of <500 ml/day.

#### **CONCLUSION & RCOMMENDATION:**

Considering the significance and proven role of microalbuminuria in the detection of early renal impairment in diabetes mellitus, it is recommended that screening for MA should be incorporated into the management of DM patients with associated risk factors for MA. The risk factors are similar to those reported from other Asian countries. Because of the adverse impact of proteinuria on survival in subjects with type 2 diabetes, screening and intervention programs should be implemented early at the stage of microalbuminuria and risk factors should be treated aggressively.

The study have shown that the protein:creatinine ratio for a random urine sample might be used to rule out the presence of significant proteinuria as

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defined by a quantitative measure of the 24-h protein excretion. This test could be the reasonable alternative to the 24-hour urine sample collection for the detection of significant proteinuria in type 2 diabetes mellitus patients. When results above the cutoff value for the protein: creatinine ratio are obtained, a full 24-h [urine](#) collection and quantification are indicated. Further prospective studies will be required in specific patient populations to validate these conclusions. The findings of this study may be helpful in achieving the goals associated with screening for proteinuria in at-risk populations.

UNDER PEER REVIEW

TABLE NO. 01: BASELINE AND CLINICAL CHARACTERISTICS OF STUDY

## SUBJECTS

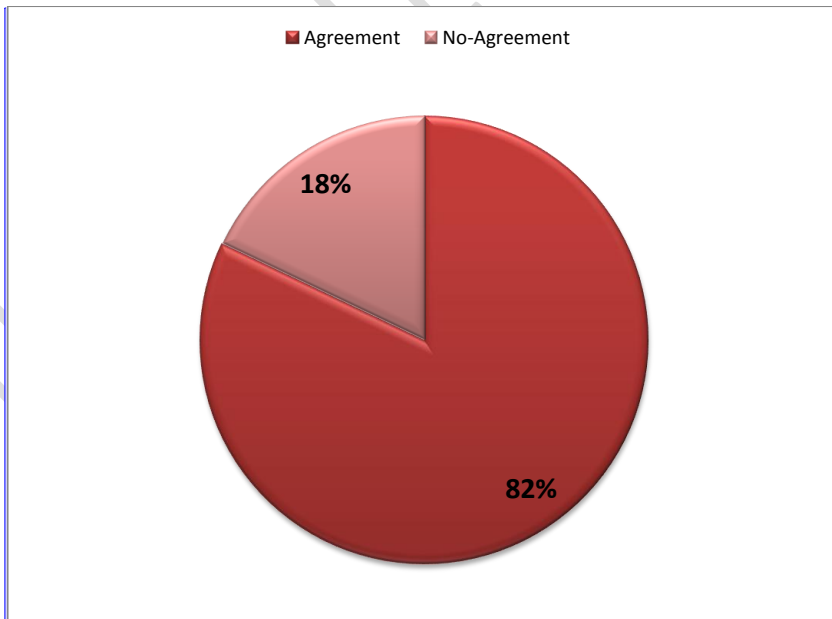
(N = 95)

Baseline Characteristics	N (%)
	(N = 95)
<b>Age – years</b>	
Mean age $\pm$ SD	41.91 $\pm$ 14.29
<b>Range</b>	
<40	40 (42.1)
>40	55 (57.8)
<b>Gender</b>	
Male	66 (69.4)
Female	29 (30.5)
<b>Marital Status</b>	
Married	62 (65.2)
Single	33 (34.7)
<b>Area of residence</b>	
Urban	68 (71.5)
Rural	27 (28.4)
<b>Clinical Characteristics</b>	
<b>Level of HbA1c - %</b>	
Mean age $\pm$ SD	6.18 $\pm$ 0.99

<6.5	36 (37.8)
>6.5	59 (62.1)
<b>Microalbuminuria</b>	
Yes	30 (31.5)
<b>Macroalbuminuria</b>	
Yes	65 (68.4)
<b>Protein-Creatinine Ratio</b>	
Normal	29 (30.5)

**FIGURE NO. 01: PERCENTAGE OF AGREEMENT BETWEEN SPOT PROTEIN-CREATININE RATIO AND 24 HOUR URINARY PROTEIN**

(N = 95)



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**TABLE NO. 02: DESCRIPTIVE STATISTICS OF 24 HOUR URINARY PROTEIN AND  
SPOT URINARY PROTEIN CREATININE RATIO**

(N = 95)

Characteristics	Mean	SD	Range	Minimum	Maximum
24 Hour Urinary Protein	1216.99	949.51	2500	100	2600
Spot Urinary Protein	1919.12	2129.25	9366	84	9450
Spot Urinary Creatinine	1616	1052.73	4250	250	4500

**TABLE NO. 03: KAPPA STATISTICS FOR ANALYSIS OF AGREEMENT BETWEEN  
SPOT PROTEIN-CREATININE RATIO AND 24 HOUR URINARY PROTEIN**

(N = 95)

Characteristics		Spot Protein-Creatinine Ratio			
		Normal	%	Not Normal	%
24 Hour Urinary Protein	Normal	29	30.5	1	1.1
	Not Normal	0	0	65	68.4
Kappa value	Total	29 (30.5%)		66 (69.5%)	
		0.975			
p value		<0.001			

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